ABSTRACT

The differential absorption, in the hind limb and neck was evaluated in six Atlantic ridley turtles. A commonly used excretory urographic iodinated contrast material was injected subcutaneously and serial radiographs were obtained. The 5-min and 70-min films were evaluated independently by five radiologists. The data obtained were compiled and the mode, mean, and range for the hind limb and neck absorption rates were analyzed. The neck site showed more rapid absorption than did the hind limb.

Further, the area of the urinary tract was serially radiographed in these animals to evaluate whether the urographic contrast agent would opacify the tract. No opacification of the kidneys was seen on serial films made up to 2½ hr after injection.

RADIOLOGIC EVALUATION OF THE DIFFERENTIAL ABSORPTION OF DIATRIZOATE IN MARINE TURTLES

INTRODUCTION

Since September 1977, the National Marine Fisheries Service (U.S. Department of Commerce) has been raising hatchlings of the Atlantic ridley (Lepidochelys kempi) and loggerhead (Caretta caretta) marine turtles at the Southeast Fisheries Center's Galveston Laboratory. The hatchlings were raised to provide a better understanding of the early life of those animals, and to engage in a headstart program as part of an overall project attempting to help repopulate the Atlantic ridley in nature. At the time the rearing was started, the Atlantic ridley had been classified as an endangered species under the U.S. Endangered Species Act of 1973. The loggerhead was later designated "threatened," effective 6 Sept. 1978 (U.S. Dept. of the Interior, 1978a). Both species of turtles have been listed in Appendix 1 to the Convention on International Trade in Endangered Species of Wild Fauna and Flora (U.S. Dept. of the Interior, 1978b).

Of immediate concern was evaluation and treatment of various diseases found in the above marine turtles, ranging from dermatologic disorders, lung infections, bowel perforation, peritonitis, bacteremia, to emaciation. Radiologic examination was employed as a research and diagnostic modality to investigate these problems.

At necropsy the kidnevs of some turtles were found to be enlarged and edematous. Therefore, we reasoned that excretory urography might be of benefit in demonstrating abnormalities in living turtles with suspected kidney disease. Excretory uro-

graphy is based upon the use of water soluble iodinated contrast agents. These agents are administered parenterally, excreted almost exclusively by goloerular filtration, not resorbed by the tubules, and concentrated by the normal solute and water resorbing processes of the kidney, thereby providing radiographic contrast in the kidney due to the high density of iodine within it, i.e., the kidneys can be seen on radiographs, a process known radiographically as opacification (Witten et al., 1980).

The opacification technique is a well-known and widely-used radiologic procedure in humans (Witten et al., 1980) as well as in some animal species such as the dog and cat (Gillette, 1977). It has been attempted in birds but has not been successful (Altman, 1978). The preferred route of administration of contrast is intravenous. However intravenous access in small turtles, although possible, is not always successful or satisfactory, (Fry, 1978). Therefore, a repeatable, satisfactory and safe means of administration with reproducible results is needed.

Cerny and associates, in a two-phase study using sodium diatrizoate in rabbits, demonstrated that the subcutaneous administration of contrast material produced satisfactory results as well as an acceptable level of side effects and minimal tissue injury (Cerny et al., 1967). Afterward they obtained satisfactory excretory urograms in 98 of 100 human patients with subcutaneous injections, while minimal side effects and no tissue slough were observed. Further, sodium diatrizoate and similar compounds have little observed toxicity in animals (Gillette, 1977). Since the subcutaneous method of administration is a recognized safe alternative to intravenous injection, it was chosen for this study.

In a preliminary study to opacify the kidneys in two

loggerhead marine turtles, we used sodium diatrizoate, a commonly used radiographic contrast agent, injected subcutaneously either in the neck or hind limb followed by serial filming. The kidneys were not visualized after 2½ hr. The contrast agent was absorbed more quickly from the neck injection site than from the hind limb site. These absorption rates could be useful for determining injection site selection for other purposes, such as antibiotic therapy in suspected or proven infections.

In this paper we report the use of sodium diatrizoate to demonstrate differential absorption from two subcutaneous sites, the neck and the hind limb in the Atlantic ridley turtle. The kidneys were also observed to determine if opacification of the urinary tract occurred.

MATERIALS AND METHODS

After the earlier studies involving two loggerhead turtles of approximately 300 g each, six Atlantic ridley turtles from four to six months of age, and ranging in weight from 100 to 320 g, and in apparent good health were chosen at random from the rearing tanks at the Galveston Laboratory. The drug used for injection was sodium diatrizoate (50% W/V) (Hypaque^{R} Sodium 50% brand of diatrizoate sodium injection, USP sterile aqueous injection, Winthrop Laboratories, 90 Park Avenue, New York, N.Y. Reference to trade names or commercial companies does not imply endorsement by the National Marine Fisheries Service, NOAA). Sodium diatrizoate is freely miscible with blood, absorbs x-rays in part of the diagnostic x-ray spectrum (due to its high atomic number), and is therefore opaque (Blaufox and Freeman, 1978). A dosage of 150 mg (0.5 cc of sodium diatrizoate 50%) for turtles

ranging in weight from 266 to 320 g and a dosage of 75 mg (0.25 cc of sodium diatrizoate 50%) in the two turtles weighing approximately 100 g were given (less than 85% of maximum animal dose) (Gillette, 1977). The contrast material was administered with disposable plastic syringes, using 26-gauge needles. All radiographic exposures were made on standard radiologic equipment available in the Department of Radiology, University of Texas Medical Branch, Galveston, Texas, using standard radiographic techniques, regular radiographic cassettes (Kodak X-omatic^{TM} regular cassette, Eastman Kodak Co., Rochester, N.Y.) equipped with high-speed intensifying screens (Kodak X-omatic^{TM} regular intensifying screens, Eastman Kodak Co., Rochester, N.Y.) and standard medical radiographic film (Dupont Cronex^{R} Four safety film, E. I. Dupont de Nemours and Co, Photo Products Dept., Wilmington, De.). (Table 1).

On each day of the study, two healthy ridley turtles were selected and were injected subcutaneously (with sodium diatrizoate), one in the ventral surface of a hind limb and the other in the dorsal aspect of the neck after sterile preparation of the overlying skin with an iodophor solution. Satisfactory injection was determined by two criteria: little or no leak of contrast through the needle track after the needle was withdrawn, and after the 5-min film, the retention by the opacified area of a nearly round or ovoid shape with little dissection along fascial planes, thus allowing for a similar volume and surface area for absorption. If the criteria were met, filming was continued for 15, 30, and 60 min after injection. The films on each animal were made in the dorsol-ventral projection with the radiographic technique detailed in Table 1. Upon completion, all studies were reviewed by five radiologists and were graded on the

change in size of the opacified area and decrease in the opacity of the contrast material, using the 5-min and 60-min films. The grading was done on a scale of 1 to 6 in the following manner: 1-unchanged, 2-mild, 3-moderate, 4-marked, 5-nearly complete absorption, and 6-no contrast detected. The data were then compiled into a frequency distribution curve and the mode, range, and mean determined.

RESULTS

The range, mode, and mean for the change in the size of the opacified area for the neck injections are given in Table 2 and for the hind limb injections in Table 3. The mode for the change in size of the contrast area was 2 (mild) and 4 (marked) for the hind limb and neck, respectively, with the neck injection mean being 3.9 and the hind limb being 2.5. The change in opacity of the area showed similar results with a mode of 3 (moderate) and 5 (nearly complete absorption) for hind limb and neck, respectively, with the mean being 4.3 for the neck and 2.9 for the hind limb. The kidneys were never observed on any of the radiographs.

We observed no untoward side effects in these animals, and there was no evidence of tissue necrosis or cutaneous sloughing at the injection sites. Radiation exposure was of concern. However the total dose was approximately 6.8 milliroentgen per exposure at 65 kVp and 2 MAS at a distance of 90 cm. Measurements were made using a 15 cc pancake ionization chamber (Keithley 15 cc pancake ionization chamber, Keithley Co., Cleveland, Oh.) on a digital dosimeter (Keithley 35055 digital dosimeter, Keithley Co., Cleveland, Oh.). This is well below the LD_{50} in some chelonians shown by Cosgrove (Cosgrove, 1971). It is considerably less than that used in turtle reproductive studies by Gibbons and Green, (1979).

DISCUSSION

Although the initial studies on the loggerhead turtles failed to opacify the urinary tract, they did show nearly complete absorption of contrast material from the neck and moderate absorption from the hind limb at 1 hr. We believed this to be an important finding when considering the need to attain the highest possible blood levels of therapeutic agents in turtles that are known to have or are suspected to have serious or life threatening systemic or localized disease amenable to drug therapy. Traditional parenteral drug administration in sea turtles is often done by injection into the hind limb area. Results in the present study indicated that subcutaneous injection in the dorsal neck is perhaps more desirable in order to attain maximal therapeutic effects.

Vascular access in these animals is quite limited, and repeated subcutaneous or intramuscular injections become necessary. The absorption of drugs from either of these sites depends on several factors. Vascularity, blood flow, solubility, and concentration all have important roles in drug absorption (Fingle and Woodbury, 1975). Sodium diatrizoate is hypertonic and highly soluble in water. Both of these factors lend themselves to its rapid absorption. These factors, of course, are constant when the same drug and drug concentration are used at different injection sites. The vascular supply to the area and the blood flow then become predominant factors. In turtles that died for various reasons, at the Galveston Laboratory, we observed at necropsy a denser vascular network in the neck than in the limbs. We believe this directly accounts for the observed difference in absorption demonstrated in this study. In Tables 2

and 3 we show that, in general, there were marked changes from the 5-min to 1-hr films in the two parameters seen in the animals injected in the hind limb (Figs. 1 and 2). Considering these findings we believe that the faster absorption of sodium diatrizoate from the neck would result in higher blood levels of this compound and therefore of other drugs with related pharmacokinetics.

Although the absorption of any drug from a subcutaneous or intramuscular site depends on its peculiar pharmacology and the environment at the site, any of the commonly used drugs that are water soluble and do not cause unacceptable tissue damage when injected subcutaneously would be absorbed more rapidly from neck injection sites due to that site's greater vascularity as has been indirectly shown by this study. Therefore, the choice of the neck as opposed to the limb as an injection site where high levels of therapeutic agents are needed is recommended.

Unfortunately, the urinary tract was not opacified in these animals. This may be due to many factors including inadequate dose, peculiar distribution, and metabolism of contrast material in these animals, and inadequate filming time (though filming was carried out to 2½ hr in the preliminary studies with the loggerhead turtles).

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The investigations involving the ridley turtle were conducted under Permit No. PRT. 2-1770 (U.S. Federal Fish and Wildlife Service), Scientific Permit No. 359 (Texas Parks and Wildlife Service), Permits 1978-ABC-IV0751, No. 27611-8786, and 1979-ABC-IV-1258, Exp. 4287 (Mexican Government).

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Figure 1. Enlargement of the hind limb area of an Atlantic ridley turtle at 5-min and 1-hr post injection. The arrowheads point to the opacified area. Comparison of the 5-min and 1-hr films show that a substantial quantity remains at 1 hr (turtle weight 310 g and carapace length 12 cm).

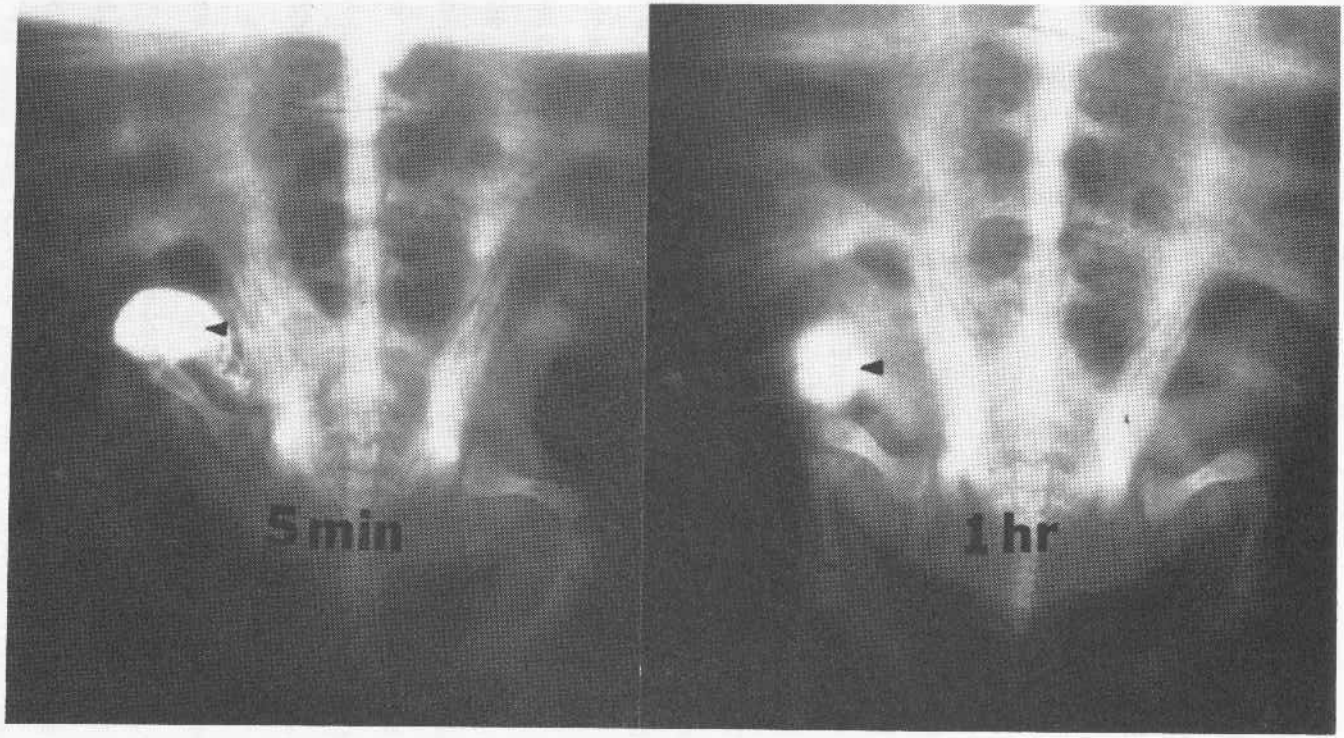

Figure 1

Figure 2. Enlargement of the neck of an Atlantic ridley turtle at 5-min and 1-hr post injection. The arrowheads point to the opacified areas. Comparison of the 5-min and 1-hr films show that at 1 hr the contrast material is only faintly visible and has been almost completely absorbed (turtle weight 100 g and carapace length 8.4 cm).

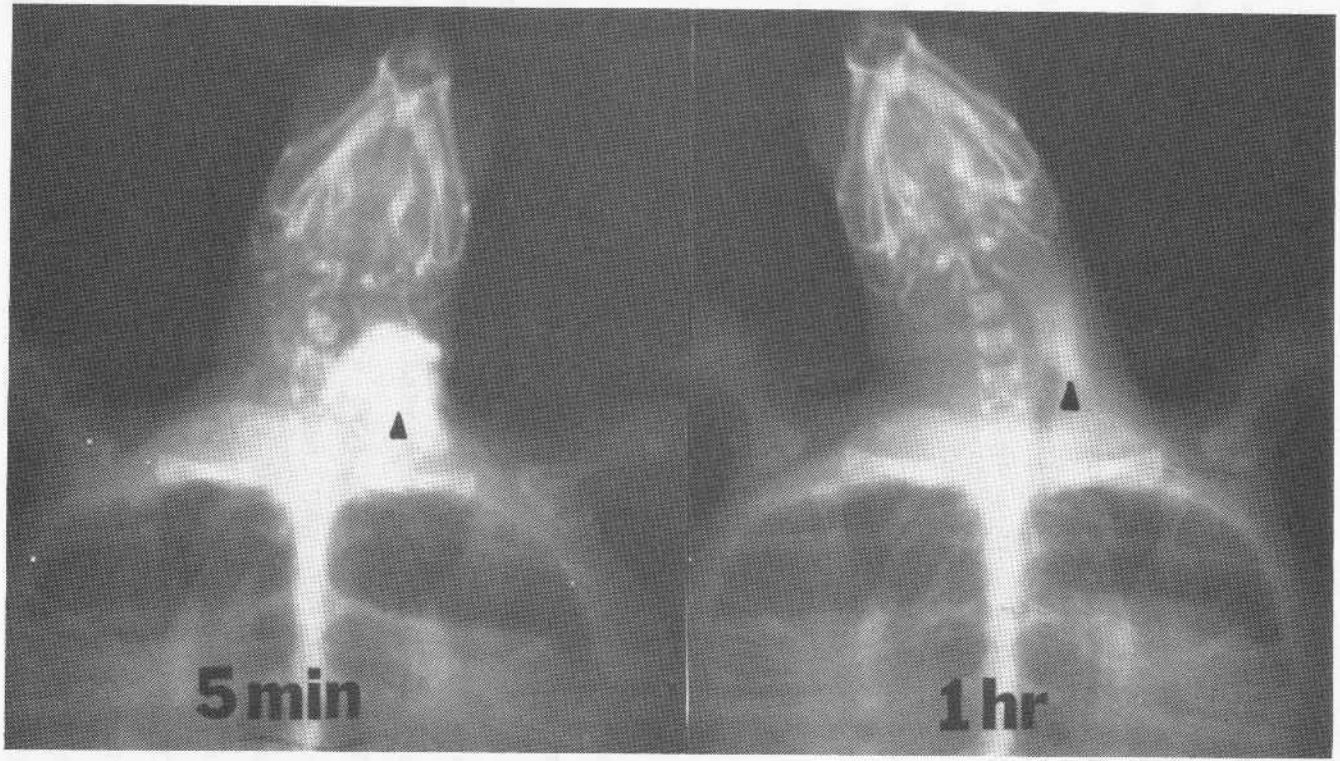

Figure 2

Table 1. Radiographic Technique. Kilovoltage peak mass 65. Milliampere seconds was 2, with a tube film distance of 90 cm.

Turtle Number	Injection Site	Turtle Weight (g)	Turtle Carapace Length (cm)	Turtle Dorso-ventral Thickness (cm)
446	Hind Limb	266	11.3	4.0
465	Neck	320	12.2	5.2
469	Neck	275	11.6	4.5
479	Hind Limb	100	8.0	3.6
470	Neck	100	8.4	3.5
473	Hind Limb	310	12.0	4.2

Table 2. Neck Injection - Grading of Change Over 1 hour.

Change in size of contrast area		Change in opacity of area	
Grade	No. of Occurrences*	Grade	No. of Occurrences*
0	0	0	0
1	0	1	0
2	0	2	0
3	5	3	3
4	7	4	5
5	3	5	7
6	0	6	0
	Mode 4 Range 3-5 Mean 3.9		Mode 5 Range 3-5 Mean 4.3

*Total number of times the grade was selected by observers.

Table 3. Hind Limb Injection - Grading of Change Over 1 hour.

Change in size of contrast area		Change in opacity of area	
Grade	No. of Occurrences*	Grade	No. of Occurrences*
0	0	0	0
1	0	1	0
2	9	2	5
3	5	3	7
4	1	4	3
5	0	5	0
6	0	6	0
Mode 2 Range 2-4 Mean 2.5		Mode 3 Range 2-4 Mean 2.9	

*Total number of times the grade was selected by observers.